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Growth Inhibitory Activity of *Clinopodium Nepeta* Essential Oil against Gram-positive and Gram-negative Bacteria: *in vitro* Studies

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ABSTRACT

Present research reports chemical composition and antibacterial activity essential oil from aerial parts of *Clinopodium nepeta* growing widely in Mascara, Algeria. It is an ornamental plant with many medicinal usages. Antimicrobial activity of essential oil extracted by hydro-distillation process from *Clinopodium nepeta* was tested on gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria. The essential oil yield from *Clinopodium nepeta* was 0.54–0.68% (v/w), which exhibited antibacterial activity against the test bacteria displaying zone diameter of inhibition ranging from 14 – 17 mm. Thus, the plant might be an important source of active ingredients useful to combat bacterial infection to humans.

Keywords: *Clinopodium nepeta*, essential oil, antibacterial activity, gram-negative bacteria, gram-positive bacteria

1. INTRODUCTION

Medicinal and aromatic plants are used for treatment as alternative therapies (Blowman et al., 2018; Das and Mandal, 2022). Plant essential oils are useful to detoxify and to promote the human health. They are also excellent sources of natural preservatives with antioxidant potential and promising antimicrobial agents against bacterial pathogens (Mandal and Mandal, 2016). Their chemical composition includes primarily terpenoids, like monoterpenes, sesquiterpenes, diterpenes, acids, alcohols, aldehydes (Masango, 2005).

In the last few years, a worrisome increase in antibiotic resistance of pathogenic bacteria has been observed (Levy, 2000). The Bacterial resistance is a global health problem with significant impact on mortality rates and health care costs (Boucher et al., 2009**)**. Evidence has indicated that multi-drug resistant (MDR) bacterial strains can become increasingly virulent, hindering the treatment of several infections (Mandal, 2015). Resistances among gram-positive and gram-negative pathogens that cause infection in the hospital and in the community

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have been reported, and bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* have been recognized as highly pathogenic microorganisms responsible for a significant number of serious infections (Mogasale et al. 2021). Based upon the emergence of antibiotic resistant bacteria, priority in the search for new antimicrobial agents has been given for combating MDR pathogenic bacteria (Demirci et al., 2011).

In the folk medicine of different countries of the world, *Clinopodium nepeta* L. (Lamiaceae family), belonging to the mint family: Lamiaceae (with 236 genera is a large dicotyledonous group with a high number of aromatic species), has been widely used for the treatment of insomnia, depression, respiratory and gastroenteric diseases (Saltos et al., 2014). Its essential oil is extensively employed to add a distinctive aroma and flavour to food and can be used in cosmetics or pharmaceuticals for an enormous number of ailments (Chouhan et al., 2017).

The genus *Clinopodium* consist of flowering plants, widely distributed in southern and southeastern Europe, North America, Latin America and Asia. This aromatic plant, reaching 80 cm in height (Dorman et al., 2000). It has the appearance of a small shrub of lilac flowers, with small and ovals leaves and it is widely distributed in Mediterranean region (Bozovic 2017). The earlier authors have also reported the antibacterial activity of *Clinopodium nepeta* essential oil (CNEO) against both gram-negative and gram-positive bacteria, and demonstrated higher activity of the essential oil against *Bacillus cereus* and *Streptococcus sanguinis* (Ozturk et al., 2021). The present study determines the antibacterial activity of CNEO as a non-traditional raw material in pharmaceuticals for the treatment of bacterial infection.

2. MATERIAL AND METHODS

The aerial parts including leaves of *Clinopodium nepeta* were collected in Ain Fares Mascara region, and the Voucher specimens was identified and deposited at the herbarium of aromatic plants of the University of Mascara, Algeria, with the number 5620. Plant materials were dried at room temperature (20–25 °C), and were grinded. Essential oil was obtained by hydro-distillation method (Debbabi et al., 2022), using air-dried ground plant materials of *Clinopodium nepeta* using 50 g of dry leaves in 1 L of distilled water and extracted for 3h.

The essential oil was dried over anhydrous sodium sulfate and stored in a scaled vial in the dark at 4°C (Adams, 2007). The yield of CNEO was calculated using a dry weight gravimetric method. Some parameters (point of freezing, refractive index, solubility in ethanol at 90 °C, and the acidity) of CNEO were measured.

The antibacterial activity of the CNEO was determined following the method, as described earlier (Mandal et al., 2007; Sircar and Mandal (2016), using sterile paper discs (6 mm diameter) impregnated with CNEO. The bacterial strains used in the study were *Escherichia coli* ATCC 3560 (from urine), *Bacillus subtilis* ATCC 4350 (from soil), *Pseudomonas aeruginosa* ATCC 27853 (from Nasal swab), and *Staphylococcus aureus* ATCC 14990 (from rectal swab).

3. RESULT AND DISCUSSION

The hydro-distillation of *Clinopodium nepeta* produced a light-yellow color essential oil. The yield percentage of *Clinopodium nepeta* aerial parts essential oil was 0.13%, with characteristic flavour, and the leaf essential oil yields up to 0.54–0.68% (v/w). Determination of density was done by double weighing d = 0.865 g/cm^3, the optical activity N20 by polarimetry was +10.12, the refractive index n = 1.4449 by an interferometric method. Ester index was 0.78 and freezing point of essential oil was -19°C (Table 1). The CNEO demonstrated bactericidal activity against both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains (Figure 1).

Table 1. Physicochemical property of *Clinopodium nepeta* essential oil

• Specification	Parametric value
• Density D20	0.865 g/cm^3
• Refractive index	1.4449
• Optical activity N20	+10.12
• Solubility in ethanol (90 %)	1 :1
• Freezing point (°C)	-19
• Ester index	0.78

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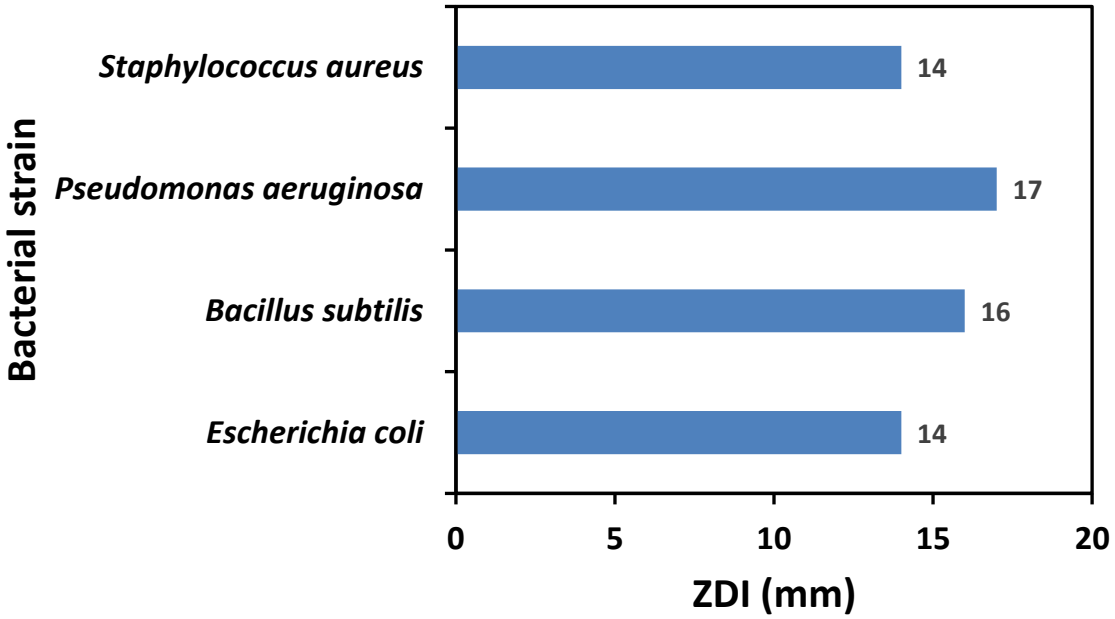

Figure 1. Antibacterial activity in terms of ZDI (zone diameter of inhibition) of *Clinopodium nepeta* essential oil against gram-negative and gram-positive bacteria

The essential oil had a strong antimicrobial activity against gram-positive as well as gram-negative bacteria (D'Aquila et al., 2022). As per the earlier report, the antibacterial susceptibility of CNEO as was determined by disc diffusion, the all microorganisms tested were sensitive to essential oil (Kitic et al., 2002). Essential oil exhibited wide-ranging inhibitory activities against various microbial pathogens and other organisms (Debbabi et al., 2022), with the penetration of lipid layer of the bacterial cell membrane thereby disrupting the bacterial cell wall structure. The various bioactive components present in the CNEO might damage the cell membrane and decrease bacterial metabolic activity (Kitic et al., 2002; Marongiu et al., 2010; Ozturk et al., 2021). *As demonstrated by* Kerbouche et al. *(2013), Staphylococcus aureus* had highest sensitivity with ZDI of 40 mm, while *Pseudomonas aeruginosa* had lowest susceptibility to the test essential oil from *Satureja calamintha* subsp. *Nepeta*. The current study provides the benefit of using CNEO as a natural food preservative by inhibiting growth of potential food borne pathogenic, such as *Staphylococcus aureus* (ZDI: 14 mm) and *Bacillus subtilis* (ZDI: 16 mm), *Escherichia coli* (ZDI: 14 mm) and *Pseudomonas aeruginosa* (ZDI: 17 mm). Thus, CNEO showed antibacterial activity against both gram-negative and gram-positive bacteria, with highest inhibition against *Pseudomonas aeruginosa*.

4. CONCLUSION

The antibacterial potentiality of *Clinopodium nepeta* essential oil suggests its capacity of usefulness in pharmaceuticals for the preparation of biotherapeutics active against pathogenic bacteria. However, analysis for bioactive compounds along with their pharmacological properties and mechanism of action are warranted, for which further research is ongoing.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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